Fungal Genetics Reports

Volume 45 Article 18

Restriction polymorphism maps of Neurospora crassa: 1998 update.

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Recommended Citation

Nelson, M. A., M.E. CRAWFORD, and D.O. NATVIG (1998) "Restriction polymorphism maps of Neurospora crassa: 1998 update.," *Fungal Genetics Reports*: Vol. 45, Article 18. https://doi.org/10.4148/1941-4765.1265

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Abstract

Restriction fragment length polymorphisms (RFLPs) can be used to determine the approximate map location of any cloned piece of DNA. To establish an RFLP mapping system for *N. crassa*, R.L. Metzenberg and coworkers crossed strains with multiple sequence differences, Oak Ridge laboratory strains (designated "O") and a Mauriceville-1c wild-collected strain (designated "M"; Metzenberg et al. 1984 Neurospora Newsl. 31:35-39; ibid. 1985 Proc. Natl. Acad. Sci. U.S.A.82:2067-2071; Metzenberg and Grotelueschen 1995 Fungal Genet. Newsl. 42:82-90). Progeny from two separate crosses have been widely distributed and used for mapping. For the first cross, 38 progeny from ordered asci were analyzed; since nonsister spores from the same half of the ascus were selected, first-division segregation can be distinguished from second-division. For the second cross only 18 random ascospore progeny were analyzed, and the small numbers limit resolution capabilities. Given this limitation, we encourage researchers to use the first cross for RFLP mapping. However, updated maps for both crosses are presented here.

Restriction polymorphism maps of *Neurospora crassa*: 1998 update.

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Restriction fragment length polymorphisms (RFLPs) can be used to determine the approximate map location of any cloned piece of DNA. To establish an RFLP mapping system for *N. crassa*, R.L. Metzenberg and coworkers crossed strains with multiple sequence differences, Oak Ridge laboratory strains (designated "O") and a Mauriceville-1c wild-collected strain (designated "M"; Metzenberg *et al.* 1984 Neurospora Newsl. **31**:35-39; *ibid.* 1985 Proc. Natl. Acad. Sci. U.S.A. **82**:2067-2071; Metzenberg and Grotelueschen 1995 Fungal Genet. Newsl. **42**:82-90). Progeny from two separate crosses have been widely distributed and used for mapping. For the first cross, 38 progeny from ordered asci were analyzed; since nonsister spores from the same half of the ascus were selected, first-division segregation can be distinguished from second-division. For the second cross only 18 random ascospore progeny were analyzed, and the small numbers limit resolution capabilities. Given this limitation, we encourage researchers to use the first cross for RFLP mapping. However, updated maps for both crosses are presented here.

Most loci are identified in the Compendium (Perkins *et al.* 1982 Microbiol. Rev. **46**:426-570) or in the more recent update to the *N. crassa* genetic maps (Perkins 1992 Fungal Genet. Newsl. **39**:61-70). Newly mapped loci included in the first cross are *nuo12.3* (NADH:ubiquinone oxidoreductase), *nuo19.3*, *nuo20.8*, *nuo21*, *nuo21.3c*, *nuo24*, *nuo29.9* and *nuo30.4* (A. Videira); pHL200, G8:1C, G8:6D, X13:5H, X13:5A, X5:9H, X16:12D, G2:3A, G7:5F, X7:8E, G12:10C and *al-2* (T.J. Schmidhauser); *mfa* (mating factor expressed in *mat a*), *rca-1* (regulat f conidiation in *Aspergillus*), *cre-1* (*A. nidulans creA* homolog), RC230.1, NP4E6, X10:E5 and X24:A11 (D. Ebbole); *cmd* (calmodulin) and *cys-9* (K. Onai and H. Nakashima); *cfp* (A.L. Rosa); *mcb* (microcycle blastoconidiation; R. Aramayo); *msh-2* (*Escherichia coli mutS* homolog; H. Bertrand); G9:12G (J. Ohmberger and R.A. Akins); *ccg-4*, *ccg-6*, *ccg-7*, *ccg-8*, *ccg-9* and *ccg-12* (J.J. Loros); *trp-4*, X17:C11, X10:A4, X4:E10, G6:E12, X25:D7, G13:C8 and G6:C9 (N.L. Glass); *aap-2* (amino acid permease), *cax* (calcium/proton exchanger), *cit-1*, *cit-2* (putative glyoxysomal citrate synthase), *vac-5* (putative 20kDa subunit of the V-ATPase), *vph-1* (vacuolar ATPase 98 kDa subunit), *vma-4*, *vma-8*, *vma-10* and *vma-11* (B. Bowman); *aod-1*, *nuo78*, *tom20* (translocase of mitochondrial outer membrane 20kDa component; formerly called MOM19), *tom22* (formerly called MOM22), *tom40* and *tom70* (F. Nargang); and *snz-1* (snooze), X11:D2, NP2G2, NP4A9, X23:C8, 3:8BL, 8:1HL, 12:11EL, 8:4GL, 12:1HL and 3:4CL (S.T. Merino, A. Errett, T. Cushing, H. Kim and M.A. Nelson). In the second cross, newly-mapped loci include *nik-2* (histidine kinase; L. Alex); *pph-1* (type-2A protein phosphatase), *rgb-1* (B regulatory subunit of type 2A protein phosphatase), *ppt-1* (serine/threonine phosphatase), *pzl-1* (phosphatase Z-like), *chs-4* and *chs-5* (O. Yarden); *aab-1* (Am Alpha Binding protein; H. Chen, J.W. Crabb and J.A. Kinsey); 31:2B, 20:5G and 26:4H (T.J. Schmidhauser); G23:3H (C.S. Enderlin and C.P. Selitrennikoff); *arg-6* (R.L. Weiss); *tef-1* (translation elongation factor 1 alpha), *mus-8*, *mus-38* and *mus-40* (H. Inoue); and G3:F4, G22:B8, G8:C8, X22:H1, G5:H2, 3:B5 and G15:B8 (S. Mohr). Several loci whose identities were previously kept confidential have been converted from five-digit numbers to informative names. Markers in the first cross beginning with AP#– or R##.#, where # is a number, are RAPD markers (Williams *et al.* 1991, pp. 431-439, in More Gene Manipulations in Fungi, J.W. Bennett and L. Lasure, Eds., Academic Press). Markers referred to as *Cen* are centromeric DNA.

In the scoring of segregants, "M" or "O" indicate segregants that are like the Mauriceville parent or the Oak-Ridge-derived parent, respectively. A dash (-) indicates that the scoring was not done or was equivocal. (O) in Iso l

and (M) in Isolate 6 of the second cross indicate parental rather than progeny strains (which are thus O and M by definition).

As with the previously published compilations, attempts were made to list the loci in map order. Several methods were employed, including computer-assisted analyses using the *Neurospora* RFLP mapping program described below and MAPMAKER version 3.0 (Lander *et al.* 1987 Genomics 1:174-181), followed by visual inspection and consideration of conventional mapping data. When possible, final placements were made to minimize double crossover and gene conversion events. However, it is inevitable that the positions of some loci will be inexact, so users should be cautious (especially when considering data from the second cross). Some anomalies of note include scores recorded as B (both) for the LG VII centromeres of segregants K4 and Q2 (first cross) and the fact that M5 possesses a morph for the centromere of LG IV that was not predicted.

We have developed *Neurospora* RFLP mapping programs for the two crosses to facilitate placement of new loci; these programs are available in on-line versions to the community through the *Neurospora* Genome Project HomePage (http://biology.unm.edu/~ngp/home.html). The RFLP mapping algorithm scores the query against each entry in the map. Each matching, resolved character (M or O), adds one to the score. Unresolved (-) characters add 0.25 to the total score. Non-matching, resolved characters add nothing to the score. (In the first cross, a score of 37.25 would be generated for 37 matching characters and one unresolved entry). The entry generating the highest score is returned. The precise placement (above or below) this match is made by comparing the scores of the entries above and below the best match and placing the query on the side with the higher score. The algorithm does not penalize (or even consider) double-crossovers or gene-conversion events.

Updated versions of the RFLP map will be available through the *Neurospora* Genome Project HomePage (http://biology.unm.edu/~ngp/home.html). An accurate and detailed RFLP map will become ever more useful as the genetic and RFLP maps converge with increasing amounts of genomic sequence information. Please share your RFLP information with Mary Anne Nelson (manelson@unm.edu) or Don Natvig (dnatvig@unm.edu). You may ask that it be assigned a number which preserves confidentiality.

Acknowledgements

The authors thank Robert L. Metzenberg, Alan Radford and David D. Perkins for consultations, and numerous researchers (cited above) for sharing RFLP data. This work was supported by the National Science Foundation RIMI (Research Improvements in Minority Institutions) Program Grant HRD-9550649 to M.A.N. and D.O.N. and NIH Grant GM47374 to M.A.N.